

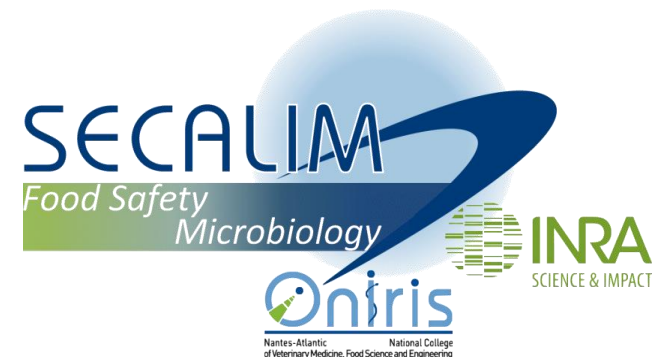


Protective Bacteria: An Option to Control *Listeria monocytogenes* in Seafood Products

Marie-France Pilet

UMR SECALIM, INRA, Oniris, 44307, Nantes, France

IAFP Athens 11-13th May 2016



Seafood products : positive aspects

High nutritional value



Unsaturated fatty acids

Vitamins

Proteins

Oligo -elements

Lightly preserved seafood products (LPSP)

- Consumption**

2008 → 166 000 t
2014 → 177 000 t

7, 3 %



Salted and/or dried fish



Cooked shrimp



READY TO EAT



Smoked fish



Surimi

Microbiology of LPSP

Spoilage bacteria

- *Hafnia alvei*
- *Phosphobacterium phosphoreum*
- *Serratia liquefaciens*
- *Brochothrix thermosphacta*
- *Lactobacillus sakei*

Economical losses

Pathogenic bacteria

- *Salmonella* spp.
- Histaminogen bacteria
- *Vibrio* spp.
- *Clostridium* spp.
- *Staphylococcus* spp.

Safety issues

Listeria monocytogenes

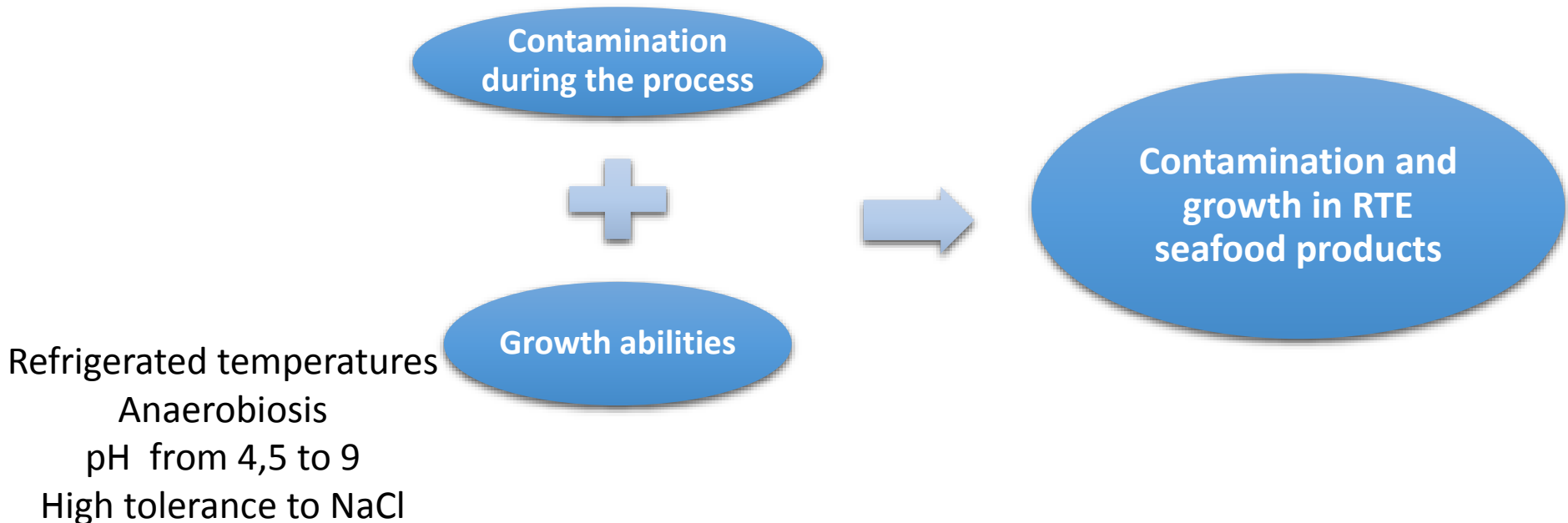
Listeria monocytogenes : a problem in seafood ?

Listeriosis

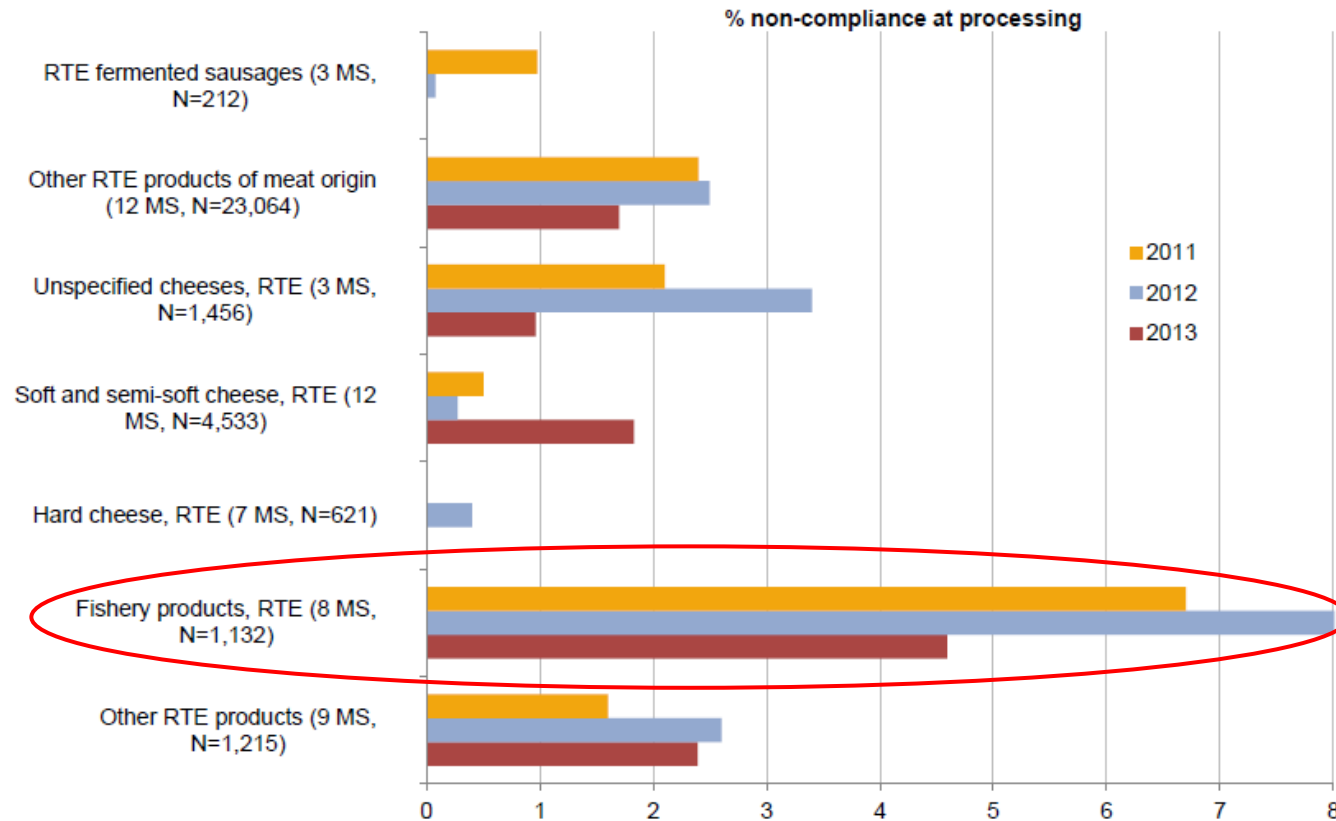
- Severe food-borne disease
- Fatality rate : 15,6 % in EU (2013)

Human listeriosis in EU

- 1763 cases in 2013
- Increasing from 2009 to 2013 : 0.37 to 0.44 cases/100 000 population



Listeria monocytogenes : a problem in seafood ?



**Fishery products :
highest levels of non
compliance**

**In 2013 : 10.8% of fish
samples positive for *L.
monocytogenes***

1.6% at level ≥ 100 CFU/g

Main hurdles in lightly preserved seafood products

Mild process

Raw or mild cooking

Salting

Preservatives

Refrigerated storage under
MAP or VP



Smoking

Shelf-life > 5 days

Fresh fish under
MAP :
8-10 days

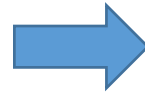
Cooked shrimp
under MAP :
12-14 days

Cold smoked fish
under VP :
21 to 42 days

Biopreservation as complementary or alternative hurdle ?

Protective lactic acid bacteria in seafood products ?

Culture-dependant and culture-independent methods
(TTGE, 16S rDNA metasequencing)



Lactic Acid Bacteria :
dominant microflora at the
end of the shelf life



(Jaffrès et al., 2009 ; Macé et al., 2011 ; Chaillou et al., 2014)



Cold smoked salmon
under VP



Cooked peeled
shrimp under MAP



Fresh salmon fillets
under MAP

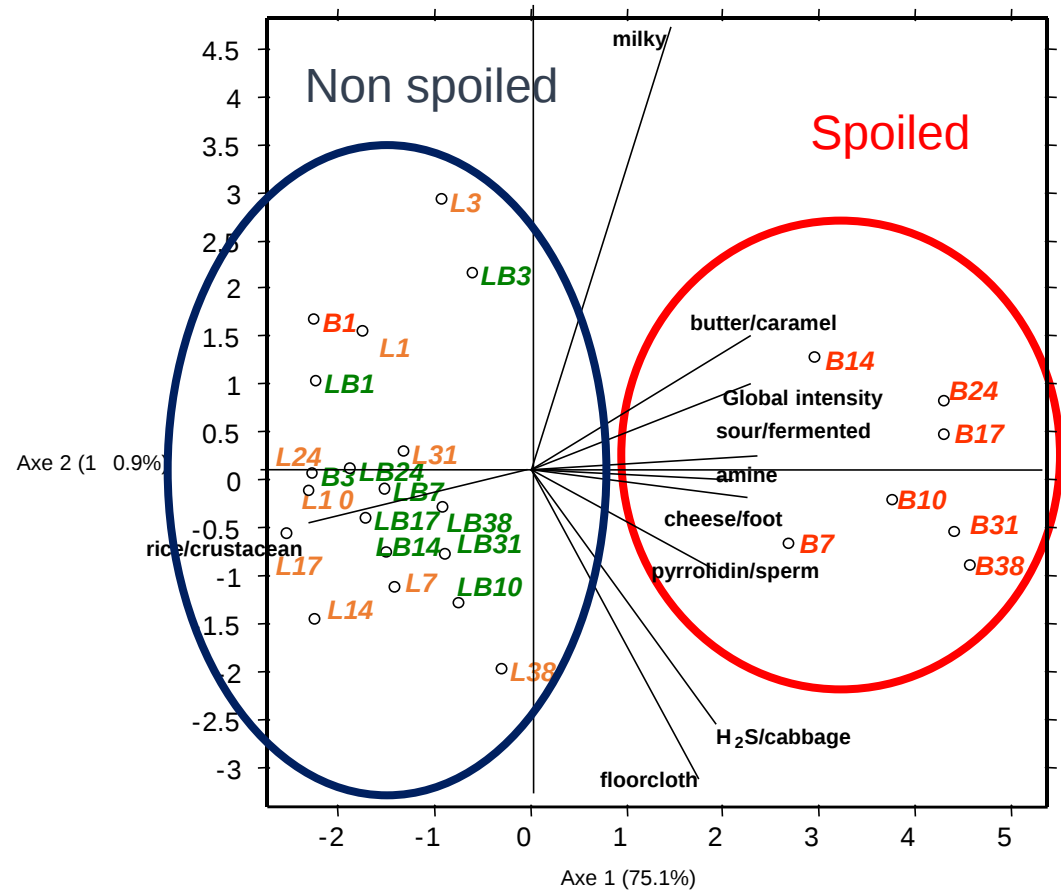
Carnobacterium maltaromaticum
Carnobacterium divergens
Lactococcus piscium

Leuconostoc gasocomitatum/gelidum
Lactobacillus sakei
Vagococcus fluvialis

Specifications for seafood protective cultures selection

- Growth on seafood matrix
- Psychrotrophic behavior
- No impact on sensorial properties of seafood

→ *Sensory analysis with trained panels*



Cold smoked salmon : a model for biopreservation studies

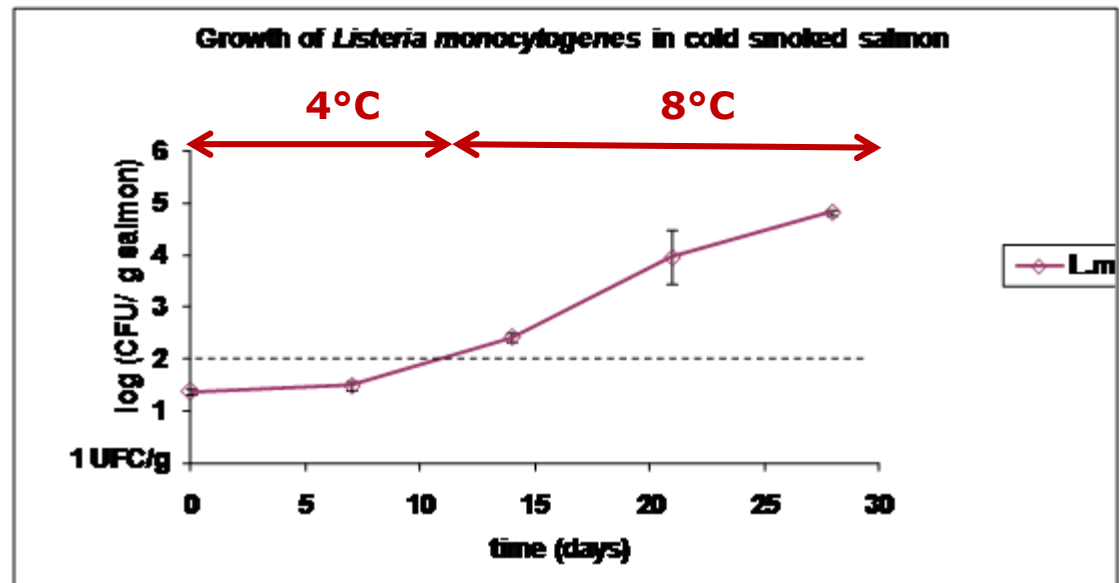


Contamination by *L. monocytogenes*:

- Raw salmon
- During the process : slicing, salting...

Growth of *L. monocytogenes*

- No bactericidal step
- Low inhibiting effect by salting (<3%) and smoking (< 1 mg phenol/100 g)
- No preservatives



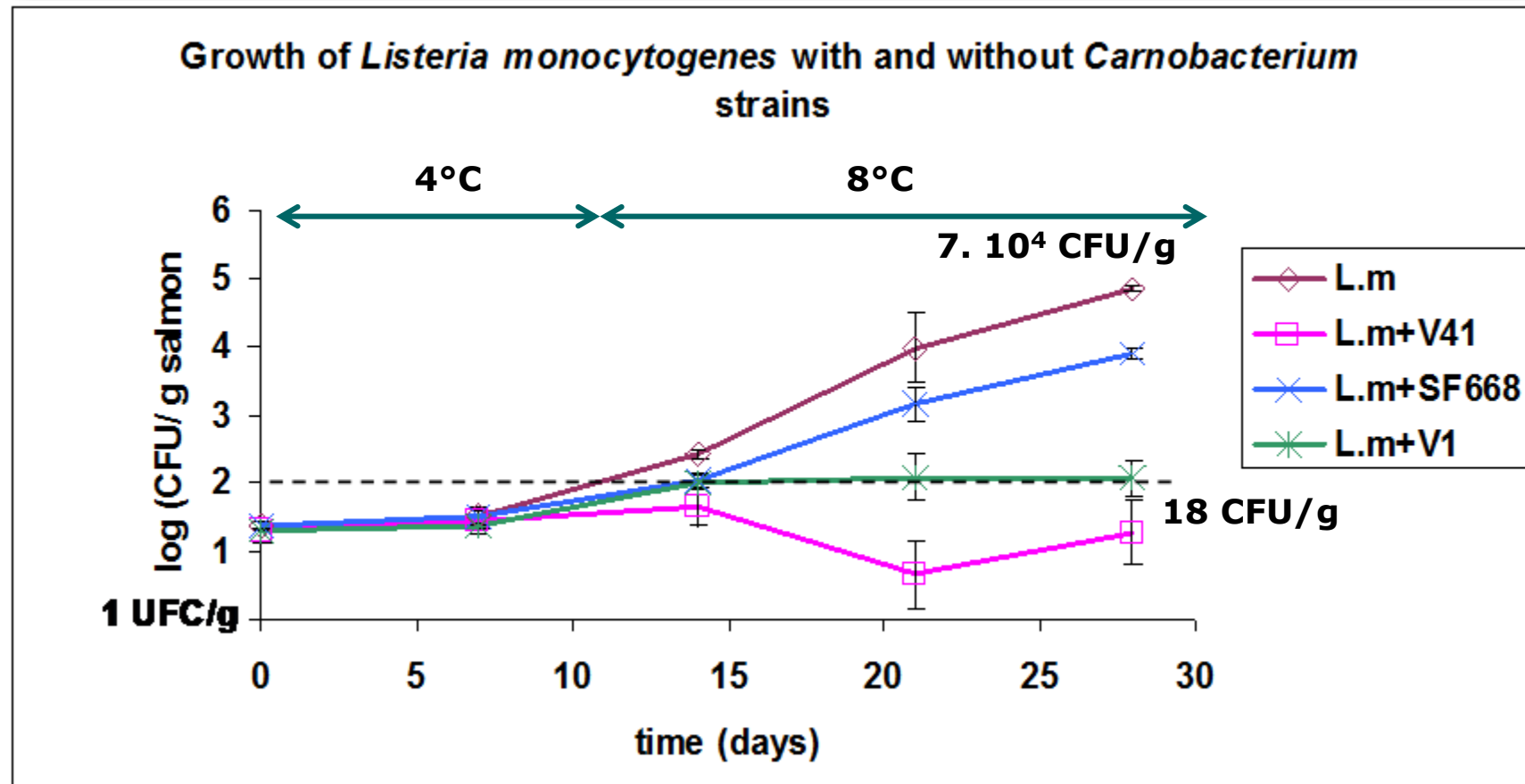
Biopreservation studies in cold smoked salmon

Protective bacteria	Inhibition mechanism	References
<i>Carnobacterium piscicola</i> A9b	carnobacteriocin B2	Nilsson et al. (2004)
<i>Carnobacterium divergens</i> V41	divercin V41	Brillet et al. (2004)
<i>Carnobacterium piscicola</i> * V1	piscicocin V1	Brillet et al. (2004)
<i>Carnobacterium divergens</i> M35	divergicin M35	Tahiri et al. (2009)
<i>Carnobacterium maltaromaticum</i> CS526	piscicocin CS526	Yamazaki et al. (2003)
<i>Lactobacillus sakei</i> Lb790	sakacin P	Katla et al. (2001)
<i>Carnobacterium piscicola</i> * A9B	Competition for glucose	Nilsson et al. (2005)
<i>Lactobacillus sakei</i> Lb790/pMLS114	sakacin P	Aasen et al.(2003)

**maltaromaticum*

Biopreservation of cold smoked salmon with carnobacteria

CHALLENGE-TEST WITH 3 BACTERIOCIN-PRODUCING *CARNOBACTERIUM* : *C. maltaromaticum* V1 and SF668, *C. divergens* V41



Biopreservation of cold smoked salmon with carnobacteria

C. divergens V41 : additional hurdle to limit *L. monocytogenes* growth in cold smoked salmon

- Inhibition effect : 4 log CFU/g
- No effect of the PC on the sensory properties of the salmon (trained panel)
- No significative modification of physico-chemical parameters : pH, TVBN...
- Slight effect on endogenous microflora (enterobacteria)
- Safety aspects : antibiotic resistance, histamine negative, no cytotoxicity
- *Cb divergens* included in the QPS list in 2014

Inhibition by *Cb. divergens* V41 : mechanism

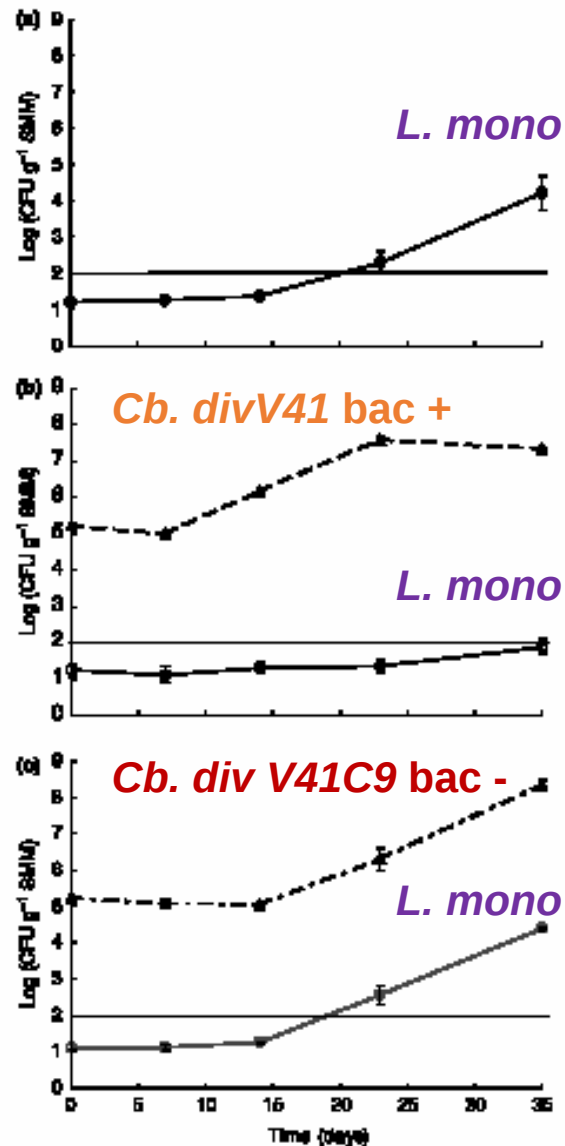
Mutant deficient in
bacteriocin production

Cb. divergens V41C9



Challenge-test in cold
smoked salmon

Comparison with Bac+
strain

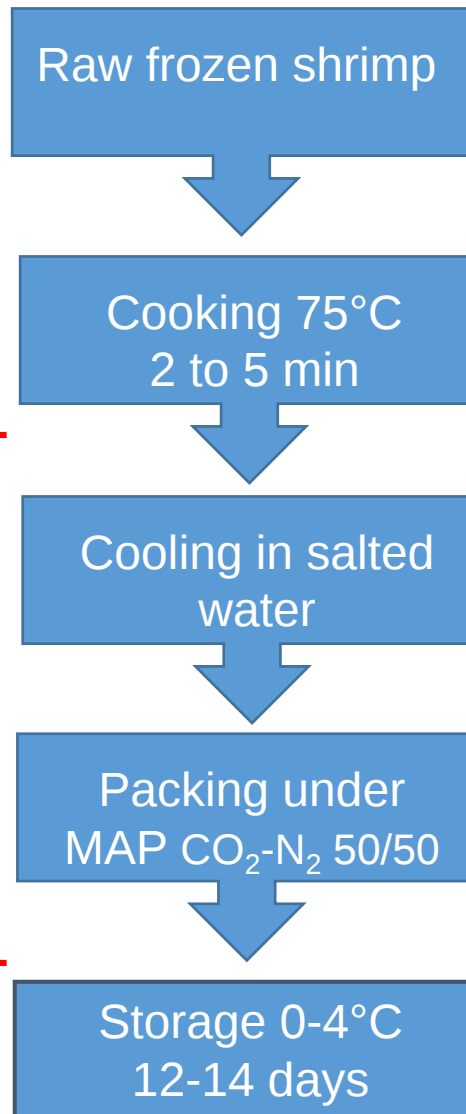


Role of bacteriocin
production /strain
growth

No durable effect of
semi-purified
bacteriocin

Nutritional
competition or
other mechanism ?

Biopreservation studies in cooked shrimp



Main hurdles for *L. monocytogenes* :

➤ Preservatives (organic acids)

***Protective culture as
alternative ?***

**! Contamination by
*L. monocytogenes***

Protective culture selection for shrimp biopreservation

Lactococcus piscium CNCM I-4031

T_{max} : 29°C



Isolated from fresh salmon fillets under MAP

Limitation of bacterial spoilage

Inhibition of food-borne pathogens

Growth inhibition

Metabiosis

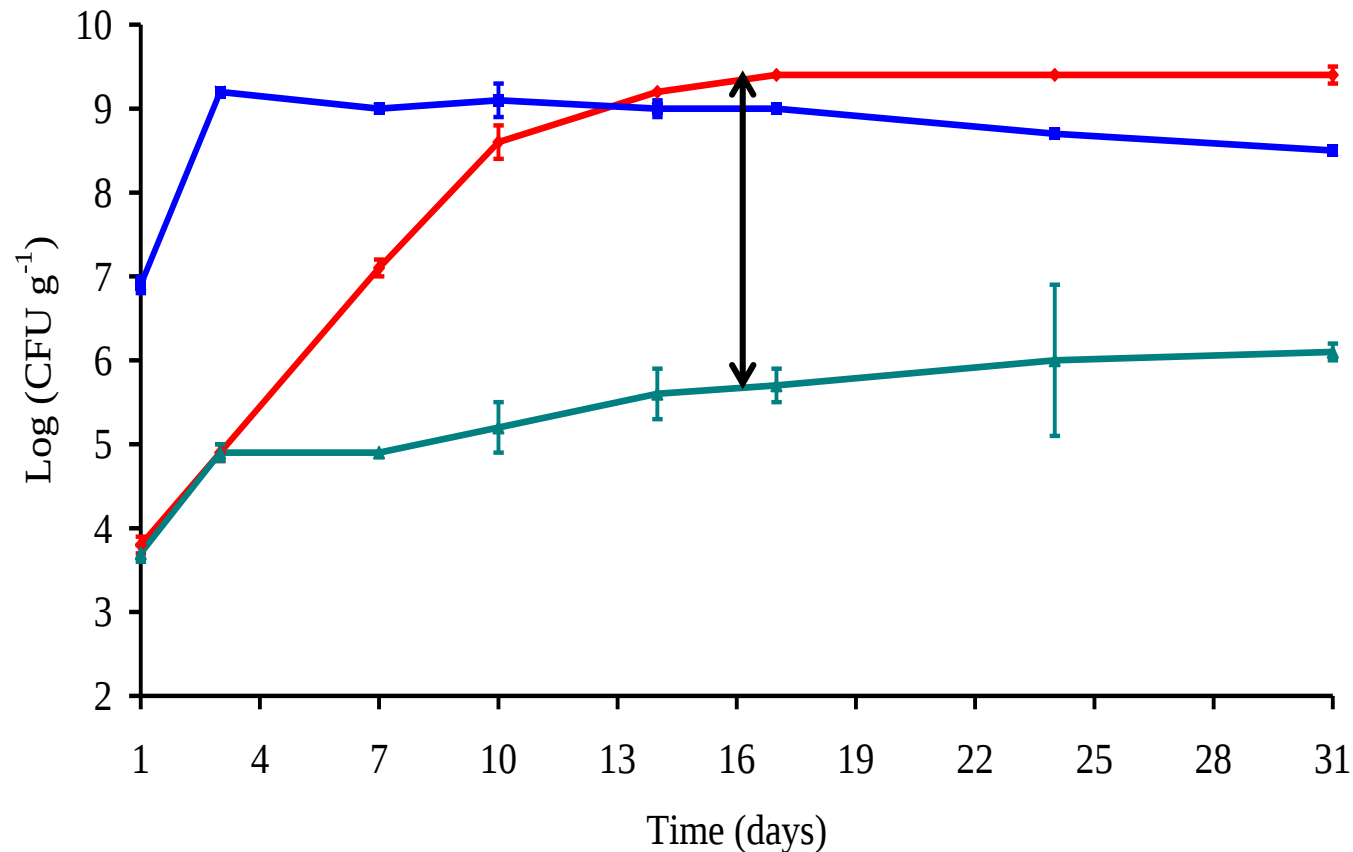
Listeria monocytogenes

Brochothrix thermosphacta
Serratia proteamaculans

Photobacterium phosphoreum
Carnobacterium divergens

Biopreservation of cooked peeled shrimp under MAP

CHALLENGE-TEST WITH *Lactococcus piscium* CNCM I-4031 at 8°C



4 log inhibition

**No modification
of the sensorial
properties**

**Safety
requirements**

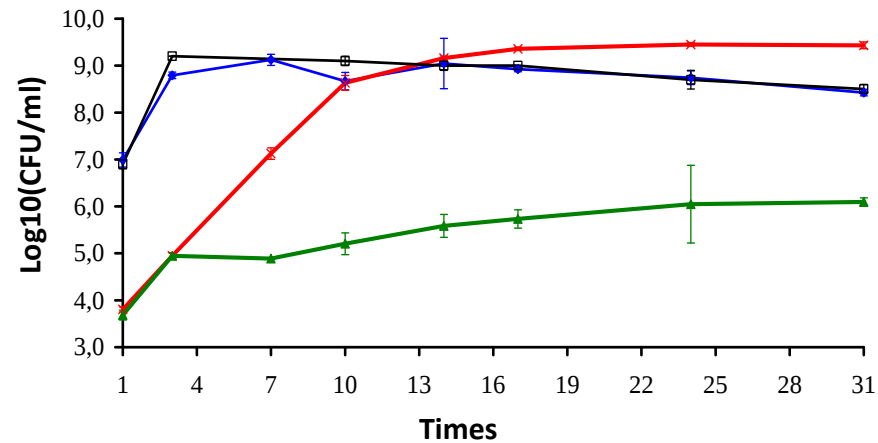
**No bacteriocin
detected in
supernatant**

—♦— *L. monocytogenes* —■— *Lactococcus piscium* CNCM I-4031 in co-inoculation —▲— *L. monocytogenes* in co-inoculation

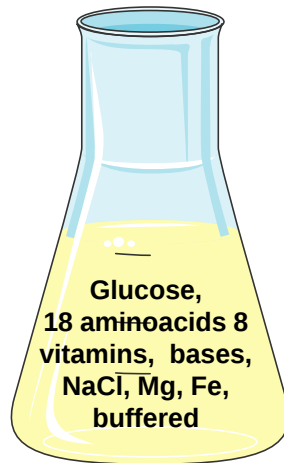
Mode of action of *L. piscium* CNCM I-4031



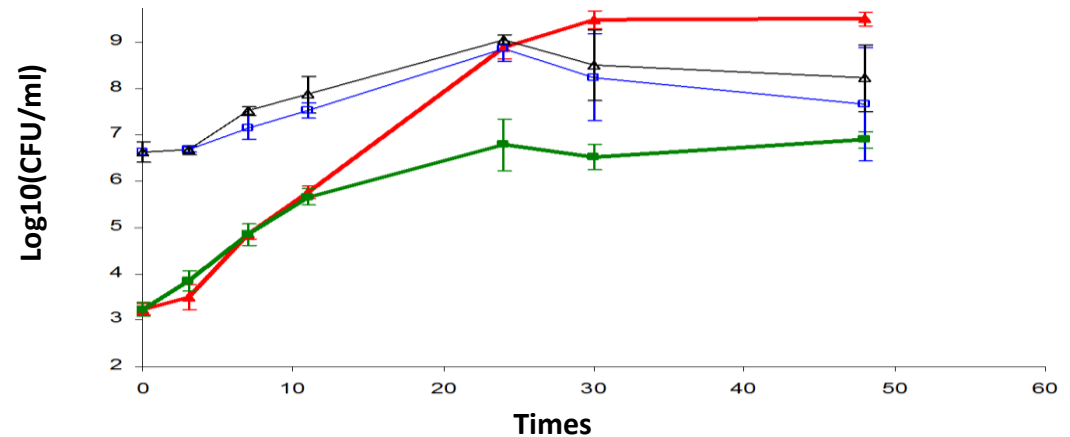
Shrimp



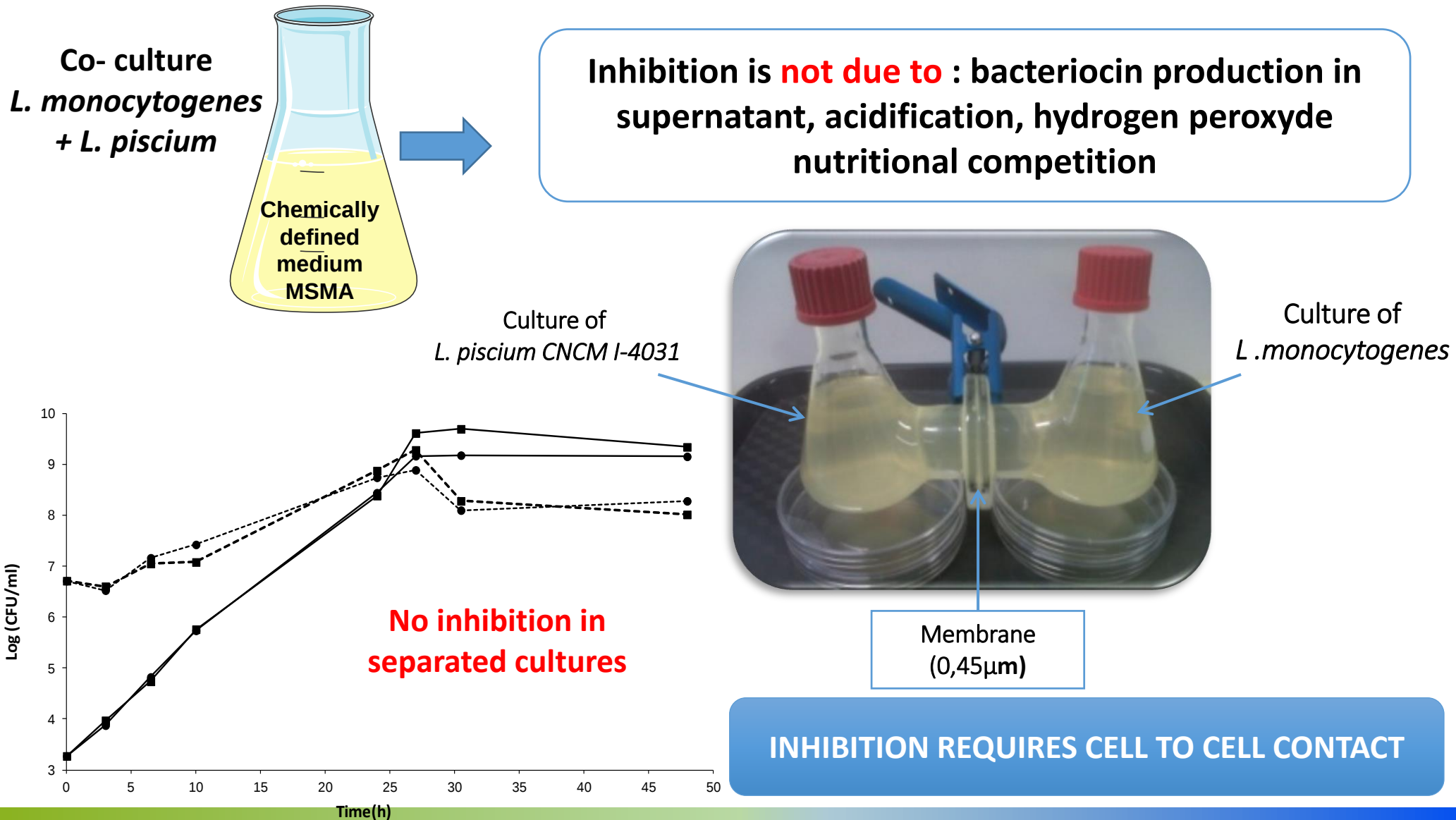
Reproduction of inhibition conditions in a chemically defined medium at 26°C



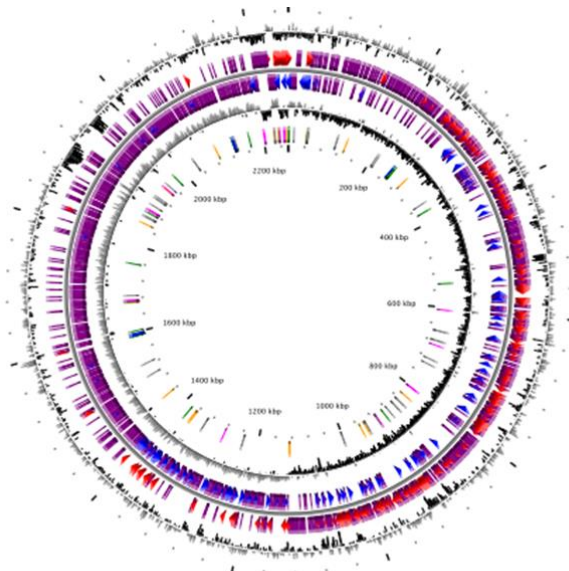
Synthetic medium



Mode of action of *L. piscium* CNCM I-4031



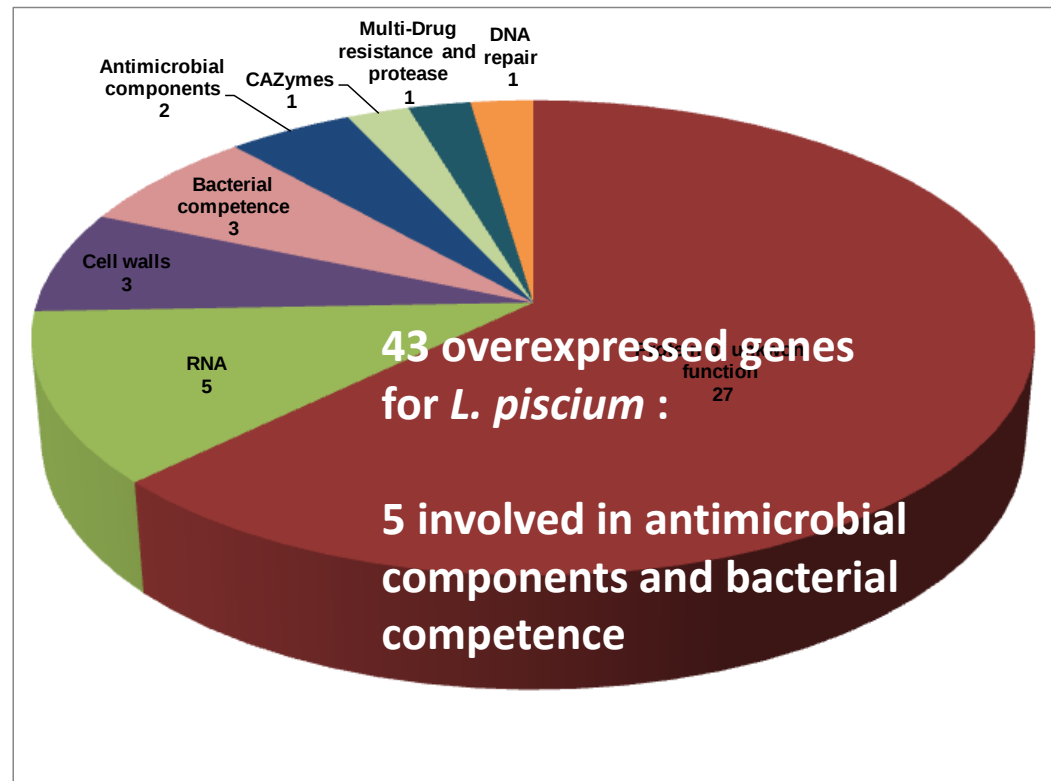
Mode of action of *L. piscium* CNCM I-4031 : hypothesis



Lactococcus piscium CNCM I-4031

Surface bacteriocins ?

Genome sequencing of *L. piscium* CNCM I-4031
RNA Seq in monoculture and 25h co-culture
conditions (max inhibition)



Conclusions

Protective lactic acid bacteria efficient to control *L. monocytogenes* in seafood : *Carnobacterium*, *Lactococcus*, *Leuconostoc*

- Few commercial applications : LLO, Lyoflora

Effect of PC on sensorial properties, metabiosis effects

- PC characterization
- Bacterial ecosystem studies and interactions

Safety aspects and action mechanisms

- To be developped with genomic and « omics » approaches

Combination with other preservation methods

- In progress : European project SafeFishDish



French network on protective culture :

- Research projects
- Regulation aspects
- Consumer perception studies



<http://www.actia-asso.eu/fiche/rmt-28-florepro.html>

Special thanks



Ifremer team : Françoise Leroi, Jean-Jacques Joffraud, Frédérique Gigout, Mireille Cardinal, Josiane Cornet

PhD students : Anne Brillet, Sébastien Matamoros, Papa Abdoulaye Fall, Taous Saraoui

Teams of the Microbiology Lab from SECALIM and IFREMER, members of the sensory panel from IFREMER...

Our partners and financial supports





THANKS FOR YOUR ATTENTION

@ marie-france.pilet@oniris-nantes.fr

@InraSecalim

<http://www6.angers-nantes.inra.fr/secalim>

SECALIM

*Food Safety
Microbiology*

Oniris

Nantes-Atlantic
National College
of Veterinary Medicine, Food Science and Engineering

INRA
SCIENCE & IMPACT